American Journal of Transplantation 2015; 15: 395–406
Wiley Periodicals Inc.

doi: 10.1111/ajt.13060

Compared Efficacy of Preservation Solutions in Liver Transplantation: A Long-Term Graft Outcome Study From the European Liver Transplant Registry

R. Adam[1,*], V. Delvart[1], V. Karam[1], C. Ducerf[2], F. Navarro[3], C. Letoublon[4], J. Belghiti[5], D. Pezet[6], D. Castaing[1], Y. P. Le Treut[7], J. Gugenheim[8], P. Bachellier[9], J. Pirenne[10], P. Muiesan[11] and all the ELTR contributing centres, the European Liver, Intestine Transplant Association (ELITA)

[1]*Centre Hépatobiliaire, AP-HP Hôpital Paul Brousse, Inserm U 776, Univ Paris Sud, Villejuif, France*
[2]*Service de Chirurgie Générale et Digestive, Hôpital Edouard Herriot, Lyon, France*
[3]*Service d'Hépato-Gastroentérologie, C.H.U. de Montpellier, Montpellier, France*
[4]*Unité de Chirurgie Digestive, C.H.U. de Grenoble, Grenoble, France*
[5]*Service de Chirurgie Digestive, Hôpital Beaujon, Clichy, France*
[6]*Service de Chirurgie Digestive, C.H.U. de Clermont Ferrand, Clermont Ferrand, France*
[7]*Service de Chirurgie Générale et Digestive, C.H.U. Marseille, Marseille, France*
[8]*Service de Chirurgie Digestive, Hôpital ARCHET 2, Nice, France*
[9]*Chirurgie Générale, Hépatique, Endocrinienne et Transplantation, C.H.R.U. de Strasbourg, Strasbourg, France*
[10]*Abdominal Transplant Surgery, University Hospitals Leuven, Leuven, Belgium*
[11]*Queen Elisabeth Medical Center, Queen Elizabeth Hospital, Birmingham, United Kingdom*
[*]*Corresponding author: René Adam, rene.adam@ap.pbr-hop-paris.fr; rene.adam@pbr.aphp.fr*
A list with all the centres is available at the following link: http://www.eltr.org/spip.php?page=centers-tous

Between 2003 and 2012, 42 869 first liver transplantations performed in Europe with the use of either University of Wisconsin solution (UW; N = 24 562), histidine-tryptophan-ketoglutarate(HTK; N = 8696), Celsior solution (CE; N = 7756) or Institute Georges Lopez preservation solution (IGL-1; N = 1855) preserved grafts. Alternative solutions to the UW were increasingly used during the last decade. Overall, 3-year graft survival was higher with UW, IGL-1 and CE (75%, 75% and 73%, respectively), compared to the HTK (69%) ($p < 0.0001$). The same trend was observed with a total ischemia time (TIT) >12 h or grafts used for patients with cancer ($p < 0.0001$). For partial grafts, 3-year graft survival was 89% for IGL-1, 67% for UW, 68% for CE and 64% for HTK ($p = 0.009$). Multivariate analysis identified HTK as an independent factor of graft loss, with recipient HIV (+), donor age ≥65 years, recipient HCV (+), main disease acute hepatic failure, use of a partial liver graft, recipient age ≥60 years, no identical ABO compatibility, recipient hepatitis B surface antigen (−), TIT ≥ 12 h, male recipient and main disease other than cirrhosis. HTK appears to be an independent risk factor of graft loss. Both UW and IGL-1, and CE to a lesser extent, provides similar results for full size grafts. For partial deceased donor liver grafts, IGL-1 tends to offer the best graft outcome.

Abbreviations: CE, Celsior solution; ELTR, European Liver Transplant Registry; HCC, hepatocellular carcinoma; HES, hydroxyethyl starch; HTK, histidine-tryptophan-ketoglutarate solution; IGL-1, Institute Georges Lopez preservation solution; LT, liver transplantation; PEG, polyethylene glycol; TIT, total ischemia time; UW, University of Wisconsin solution

Received 14 January 2014, revised 08 August 2014 and accepted for publication 09 August 2014

Introduction

Ischemia–reperfusion injury associated with cold preservation is well known to influence transplant outcomes (1). The avoidance of this injury can ensure improvement of posttransplant graft function and survival (2). Two different ways have been explored to diminish the injury related to ischemia–reperfusion: static cold preservation with optimal solutions, and dynamic preservation devices using different temperatures ranging from 4 to 37°C (3). Machine perfusion has proven its efficacy and is routinely applied for kidney preservation (4), but is not routinely used for clinical liver preservation (5). Given the complexity of preserving hepatic function, most innovative investigations remain in the experimental domain (6–9).

Recent improvements in hepatic preservation have been focused on the development of static cold preservation solutions with different compositions (2,3), owing to the technical simplicity and clinical feasibility of this method. The modifications in the components, introduced sequentially

in the last 40 years, are based on the change of ionic composition and on the molecules aiming to reduce intracellular and interstitial edema, namely osmotic and oncotic agents (3). Preservation solutions can be classified according to potassium levels, type of oncotic agents, presence of antioxidants and of agents protecting from ischemia–reperfusion damage. Differences in the composition of the mostly used solutions are shown in Table 1.

Despite the scientific rationale directing the different compositions, their clinical relevance on liver transplant outcomes are often questioned due to a lack of properly powered, prospective randomized designed trials (5). Recently a systematic review with meta-analysis on 16 randomized clinical trials come to the conclusions that there was no good evidence of any difference in outcomes when comparing histidine-tryptophan-ketoglutarate solution (HTK) with either of the University of Wisconsin solution (UW) or Celsior solution (CE), but the authors recognized that the data were limited (10). In this context, the purpose of this study was to ascertain graft survival in a large European cohort of patients in relation to liver preservation with the four most frequently used preservation solutions: UW, Institute Georges Lopez solution (IGL-1), CE and HTK.

Methods

Study population

Between 1968 and 2012, 118 001 adult liver transplantations (LTs) were consecutively performed by European centers and collected through the European Liver Transplant Registry (ELTR). Out of them 106 701 were first LTs and since 2003, there were 43 424 first LTs, of which 42 869 used UW, CE, HTK or IGL-1 for graft preservation. The ELTR questionnaire is intentionally limited to the main variables concerning donor and recipient data, intraoperative details as well as graft and patient outcome updated every 6 months. The quality of data is routinely controlled by randomly designated audit visits of contributing centers (5–10 audits per year), which have confirmed that data from the ELTR are a reliable and credible representation of LT practice in Europe (11).

Table 1: Composition of the different cold preservation solutions compared in the study

	CE	HTK	IGL-1	UW
mOsm/L	320	310	290	320
HES	–	–	–	0.25
PEG-35	–	–	0.03	–
Na^+	100	15	120	27
K^+	15	10	25	125
Cl^-	–	50	–	–
Mg^{2+}	13	4	–	5
Ca^{2+}	0.25	0.015	0.5	
HCO_3^-	–	–		5
SO_4^-	–	–	5	4
PO_4	–	–	25	25
H_2PO_4	–	–	–	–
Histidine	30	198	–	–
Mannitol	60	30	–	–
Lactobionate	80	–	100	105
Raffinose	–	–	30	30
Glutathione	3	–	3	3
Adenosine	–	–	5	5
Glutamate	20	–	–	–

Concentrations are expressed in mmol/L, except otherwise specified.
HES, hydroxyethyl starch; PEG-35, polyethylene glycol 35 kDa.

Study design

Recipients were divided into four groups in relation to the graft preservation solution: UW (N = 24 562—57.3%), HTK (N = 8696—20.3%), CE (N = 7756—18.1%) and IGL-1 (N = 1855—4.3%). The evolution in the use of the four preservation solutions was assessed. The preservation solution fluids were chosen at the discretion of the procurement team and the ELTR questionnaire was blinded to the reason of the choice made by the teams.

The end point of analysis was graft survival in the total study population.

In addition, graft survival was assessed for each group in relation to the length of total ischemia time (TIT; $<$ or $>$12 h), to the type of graft since deceased partial liver grafts (split or reduced) may be more likely to suffer from ischemia–reperfusion injury, or to the type of indication for LT.

Statistical method

Graft survival was analyzed for all liver grafts comparing UW, CE, HTK and IGL-1 preservation groups. Analyzed recipient variables were: gender, age, blood group, BMI, hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (anti-HCV), creatinine, bilirubin, international normalized ratio (INR), Model for End-Stage Liver Disease (MELD) score, HIV serology, dialysis, United Network for Organ Sharing (UNOS) status and main diseases. Donor/graft variables were: gender, age, blood group, preservation solution and TIT (number of minutes between portal clamping in the donor and portal revascularization on the recipient). Transplantation variables were: urgency, heterotopic/orthotopic, auxiliary LT, bypass, living, domino or deceased partial grafts including reduced and splits, associated transplantation, number of LTs per center. Follow-up variables were: date of outcome, cause of graft loss, cause of death.

Missing data were excluded from the analysis without any imputation. Categorical and continuous study variables were compared using the chi-square test and the independent-samples t-test, respectively. To evaluate if preservation solutions have an impact on graft survival in separate different transplant subgroups with known different patient survivals (12), sub-groups analyses were performed for hepatocellular carcinoma (HCC) and partial grafts (split or reduced) as well as for the total cohort excluding living related LT.

Survival probabilities were estimated using the actuarial method and were compared using the log-rank test. The cutoff values for continuous variables (such as LT center volume, recipient and donor age, ischemia time) were chosen by plotting the mortality risk ratios at each value, or at pertinent interval values, for each variable. The value corresponding to the most relevant change in the risk-ratio was used for the subsequent multivariate analysis. Multivariate analysis was performed using a Cox proportional hazards model to identify independent prognostic factors of survival in all patients. For multivariate analysis, factors with a $p \leq 0.15$ at univariate analyses were included. The analysis was adjusted to the center and a stratified Cox model analysis was performed to avoid any confounding effect of the center. A $p \leq 0.05$ in the Cox model was considered statistically significant. All statistical analyses were performed using SAS, Version 9.1, software (SAS Institute Inc, Cary, NC).

Results

Use of preservation solutions in Europe

Since 1983, when data collection started routinely in the ELTR, the unique solution used in Europe for static liver preservation was Collins solution. A maximum of 450 transplants per year were performed, with its use peaking in 1987. UW was introduced in 1987 and peaked in use in 2001 at 3750 transplants/year. Alternative solutions to UW emerged in the 1990s: HTK started to be used by 1991, CE by 1998, and IGL-1 by 2003. During the study period between 2003 and 2012, UW use decreased while the use of alternative solutions increased progressively (Figure 1). The final percentages of solutions used in 2012 were: 36% for UW, 29% for HTK, 23% for CE, 10% for IGL-1 and 2% for others.

Preservation solution versus graft survival—all transplants

Considering the whole cohort of preserved liver grafts within the study period, some differences between study groups were found related to recipient and donor data or to transplant procedure.

As shown in Table 2, HTK and UW presented higher rates of female recipients ($p < 0.0001$), IGL-1 had older recipient age ($p < 0.0001$) with the higher MELD score ($p < 0.0001$) and the higher proportion of patient having dialysis ($p < 0.0001$), CE had older donor age ($p < 0.0001$) and more patients with cancer but less with fulminant hepatitis, IGL-1 and UW had longer TITs and higher proportion of partial grafts (split or reduced), but HTK presented higher rate of living donor LT. The BMI was identical in all groups.

Graft survival between the different solutions was found to be significantly different (Global Log Rank $p < 0.0001$). Overall UW, IGL-1 and CE were associated with better survival, compared to HTK (1 year: 83%, 82%, 82% vs. 77%; 5 years: 69%, 68%, 68% vs. 64%, respectively, for UW, IGL-1, CE and HTK) (Figure 2A). Further analysis demonstrated that HTK also provided significantly lower survival than the other solutions when excluding living transplantations (Figure 2B).

In addition to the preservation solutions, all variables known to affect graft survival were also submitted to univariate analysis. Prolonged TIT, patients with HCC and transplantation with a partial liver graft were found to be associated with lower graft survival (Figure 3). Given that these variables were unequally distributed in the different preservation solution groups, graft survival was concretely evaluated by comparing all the solutions in the context of each of these factors, in subgroup studies (Table 3).

Preservation solution versus graft survival in relation to TITs

As shown in Figure 3A, a significant effect of the type of preservation solution was observed on graft survival for TITs shorter than 6 h (Global Log Rank $p < 0.0001$) (at 1 year: 86%, 84% vs. 82%, 82%; at 5 years: 73%, 76% vs. 67%, 71%, respectively, for UW, IGL-1, CE and HTK). The effect was more evident within 6 and 12 h of TIT (Global Log Rank $p < 0.0001$), UW providing higher survival than the others while HTK showed the lowest survival (at 1 year: 83%, 82%, 82%, 77%; at 5 years: 70%, 67%, 68%, 63%, respectively, for UW, IGL-1, CE and HTK) (Figure 3B). This was confirmed for longer TIT (between 12 and 18 h), HTK providing lower survival than the other solutions (Global Log Rank $p < 0.0001$) (at 1 year: 78%, 84%, 80%, 69%; at 5 years: 65%, 69%, 66%, 52%, respectively, for UW, IGL-1, CE and HTK) (Figure 3C). No significant differences were observed with TIT greater than 18 h but the number of

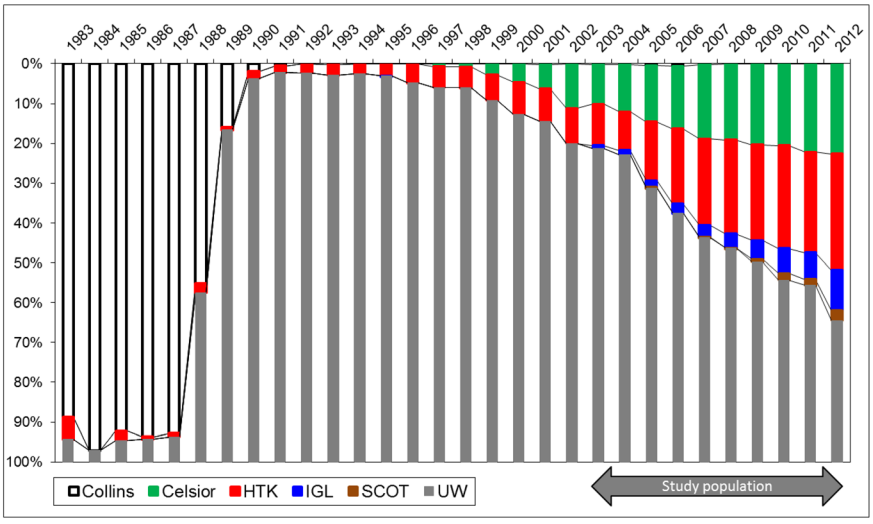


Figure 1: Use of preservation solutions in liver transplantation in Europe from 2003 to 2012. Absolute numbers.

Table 2: Demographics of patients undergoing liver transplantation

Preservation solution N	CE 7756	HTK 8696	IGL-1 1855	UW 24 562	p
Recipent characteristics					
Age (year) (mean ± SD)	53 ± 10	52 ± 11	54 ± 10	52 ± 11	<0.0001
Female (%)	27%	34%	27%	33%	<0.0001
BMI (kg/m^2) (mean ± SD)	25.6 ± 6.8	26 ± 6.3	26.6 ± 18.5	26.2 ± 22.4	0.21
Disease					
Cirrhosis	54%	60%	60%	61%	<0.0001
Cancer	31%	18%	23%	17%	<0.0001
FUHE	4%	6%	6%	7%	<0.0001
Others	10%	15%	12%	15%	<0.0001
MELD score (mean ± SD)	17.1 ± 8.9	18 ± 9.2	19 ± 10.2	17.5 ± 8.7	<0.0001
INR (mean ± SD)	1.8 ± 1.3	1.7 ± 1	2 ± 1.6	1.7 ± 1.5	<0.0001
Bilirubin (mg/dL) (mean ± SD)	5.5 ± 8.4	6.6 ± 9.5	6.6 ± 9.5	6.2 ± 9	<0.0001
Creatinine (mg/dL) (mean ± SD)	1.1 ± 0.8	1.2 ± 1	1.2 ± 1	1.2 ± 0.9	<0.0001
Dialysis	4.9%	6.9%	7.0%	2.4%	<0.0001
UNOS status (%)					
ICU-bound	7%	8%	14%	9%	<0.0001
Continuous medical care	16%	21%	13%	14%	–
Continuous hospitalization	56%	45%	50%	48%	–
At home with normal function	21%	26%	22%	30%	–
Transplant characteristics					
Total ischemia time (min) (mean ± SD)	457 ± 162	464 ± 226	477 ± 164	500 ± 180	<0.0001
Urgency (%)	6%	11%	9%	9%	<0.0001
Partial liver (split or reduced) (%)	2.8%	2.2%	5%	4.5%	<0.001
Donor characteristics					
Age (year) (mean ± SD)	54 ± 18	48 ± 17	51 ± 18	48 ± 17	<0.0001
Female (%)	43%	44%	43%	43%	0.22
Living related	1.5%	21.1%	2.3%	2.6%	<0.0001

patients was limited: UW (n = 60), IGL-1 (n = 3), CE (n = 11) and HTK (n = 30) (data not shown).

Preservation solution versus graft survival in HCC patients

All transplanted patients with HCC were selected and analyzed as a subgroup, since the incidence of HCC was different between preservation solution groups ($p < 0.001$).

Overall graft survival was significantly affected by preservation solutions (Global Log Rank $p < 0.0001$) (Figure 4). HTK showed the worst survival over time when compared to the three other ones (HTK vs. CE, HTK vs. UW, and HTK vs. IGL-1, all $p = 0.0001$). IGL-1, UW, CE and HTK graft survivals were 84%, 87%, 85%, 76%, respectively, at 1 year and 63%, 67%, 66%, 50%, respectively, at 5 years.

Preservation solution versus graft survival in patients transplanted with partial deceased grafts

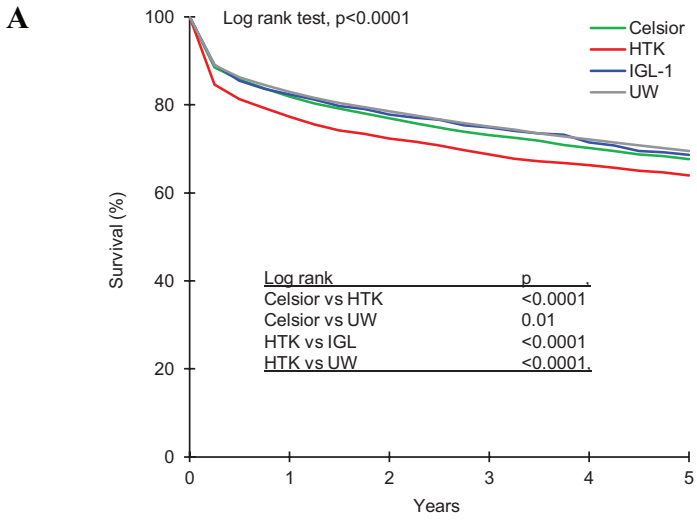
In patients receiving partial grafts, a significant difference in graft survival between preservation solutions was also demonstrated (at 1 year: 72%, 89%, 76%, 69%; at 5 years: 62%, 80%, 66%, 54%, respectively, for UW, IGL-1, CE and HTK) (Global Log Rank $p = 0.009$). As indicated in Figure 5, HTK was related to the worse survival when compared to the other solutions. IGL-1 provided a significantly better graft survival compared to HTK ($p = 0.0007$) and UW ($p = 0.001$).

Multivariate analysis of graft survival

After adjusting for centers, HTK preservation was independently associated with a 10% increased risk of graft loss on multivariate analysis when compared with CE, UW or IGL-1 ($RR = 1.10$; $p = 0.02$) (Table 4). The other factors influencing graft survival were by decreasing order of importance: recipient HIV positivity ($RR = 1.50$), donor age $\geq$65 years ($RR = 1.41$), recipient HCV positivity ($RR = 1.40$), main disease: acute hepatic failure ($RR = 1.34$), use of a partial liver graft ($RR = 1.30$), recipient age $\geq$60 years ($RR = 1.29$), no identical ABO compatibility ($RR = 1.24$), recipient HBsAg negativity ($RR = 1.24$), TIT $\geq$ 12 h ($RR = 1.19$), male recipient ($RR = 1.10$) and main disease other than cirrhosis ($RR = 1.09$).

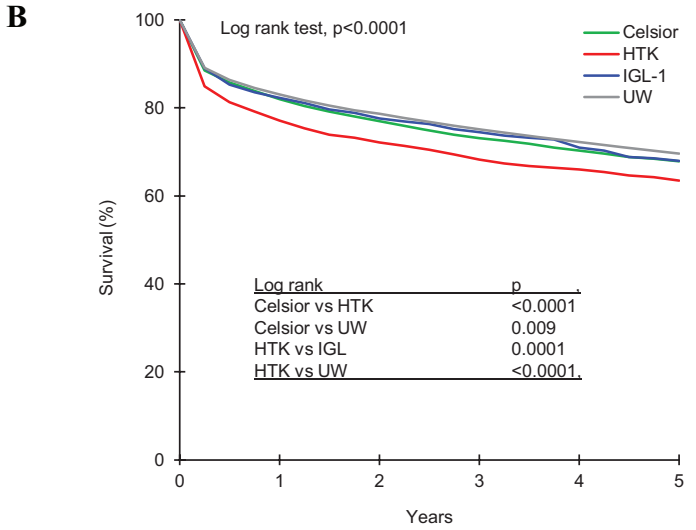
Cause of death or graft loss in relation to preservation solutions

Considering hepatic complications, the proportion of graft loss related to primary nonfunction or dysfunction (13.7%) and to biliary complications (6%) were the highest in the HTK group, as well as graft loss related to infectious complications (19.8%) (Table 5). Vascular complications (9.1%) and rejection (4.7%) were the highest with UW, and



	1 yr	2 yrs	3 yrs	4 yrs	5 yrs
Celsior	82%	77%	73%	70%	68%
HTK	77%	72%	69%	66%	64%
IGL	82%	78%	75%	71%	68%
UW	83%	79%	75%	72%	69%

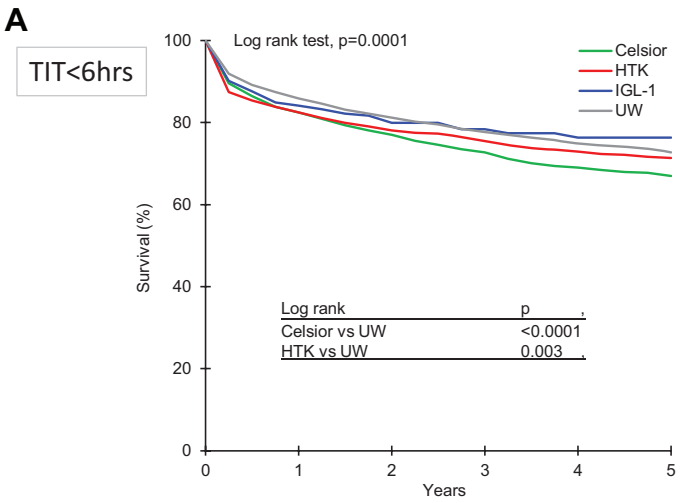
Exposed patients					
Celsior	4,684	3,458	2,653	1,947	1,335
HTK	4,238	2,974	2,098	1,430	963
IGL	1,043	717	504	351	225
UW	16,588	13,889	11,470	9,286	7,225



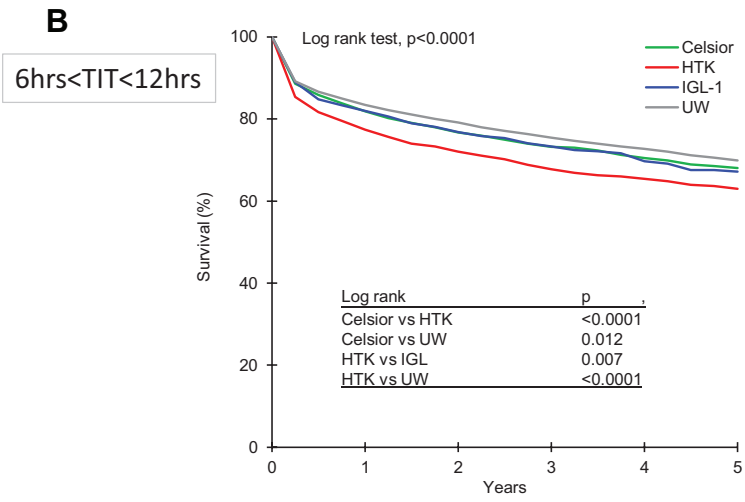
	1 yr	2 yrs	3 yrs	4 yrs	5 yrs
Celsior	82%	77%	73%	70%	68%
HTK	77%	72%	68%	66%	63%
IGL	82%	77%	74%	71%	68%
UW	83%	79%	75%	72%	70%

Exposed patients					
Celsior	4610	3404	2604	1909	1309
HTK	3464	2470	1739	1160	778
IGL	1012	689	480	329	204
UW	16258	13611	11245	9099	7070

Figure 2: (A) Graft survival after liver transplantation according to preservation solution in all patients. (B) Graft survival after liver transplantation according to preservation solution excluding living transplantation.

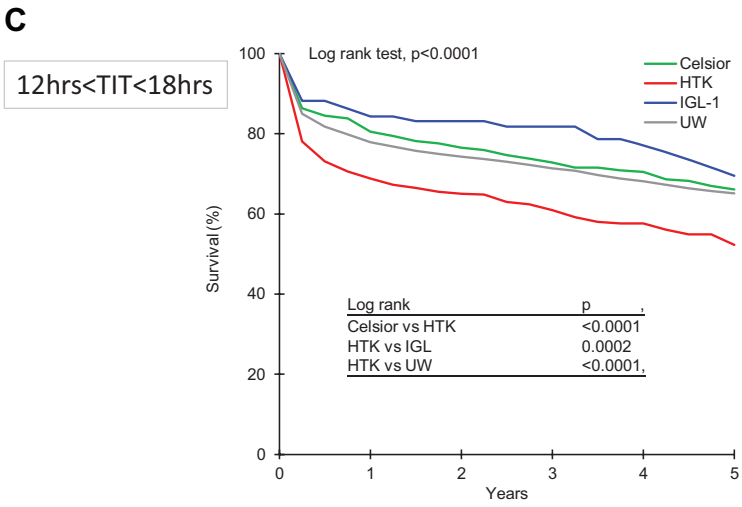


	1 yr	2 yrs	3 yrs	4 yrs	5 yrs
Celsior	82%	77%	73%	69%	67%
HTK	82%	78%	75%	73%	71%
IGL	84%	80%	78%	76%	76%
UW	86%	81%	78%	75%	73%
Exposed patients					
Celsior	1224	896	654	473	318
HTK	1162	840	637	476	349
IGL	201	135	88	64	41
UW	3619	3010	2462	2002	1599



	1 yr	2 yrs	3 yrs	4 yrs	5 yrs
Celsior	82%	77%	73%	70%	68%
HTK	77%	72%	68%	65%	63%
IGL	82%	77%	73%	70%	67%
UW	83%	79%	75%	73%	70%
Exposed patients					
Celsior	2882	2189	1691	1223	848
HTK	2346	1645	1139	743	478
IGL	744	508	359	238	151
UW	10455	8738	7215	5841	4515

Figure 3: Graft survival after liver transplantation according to preservation solution in different subgroups of grafts with different total ischemia time.



	1 yr	2 yrs	3 yrs	4 yrs	5 yrs
Celsior	80%	76%	73%	71%	66%
HTK	69%	65%	61%	58%	52%
IGL	84%	83%	82%	77%	69%
UW	78%	74%	71%	68%	65%

Exposed patients					
Celsior	326	267	231	195	142
HTK	409	290	188	113	74
IGL	82	67	55	47	31
UW	1734	1506	1308	1102	867

Figure 3: (Continued)

those related to hemorrhage (3.1%) were the highest with IGL-1.

Discussion

By analyzing a large cohort of first LTs performed in a 10-year period, this retrospective study shows that UW considered for long the gold standard preservation solution, has been less frequently used in Europe in the recent years and currently alternative solutions are used in 64% of LT. This study shows that compared to UW, graft survival is at least equally preserved with the use of IGL-1, slightly decreased with CE but significantly reduced with HTK compared to the three other solutions. In subgroups of transplanted patients with factors known to adversely affect the graft survival, the data confirm that HTK provides significantly lower survival than CE, IGL-1 and UW for HCC patients, for TIT exceeding 6 h, for partial deceased grafts and for the total population excluding living related transplantations, more widely used with HTK. Multivariate analysis confirmed HTK as an independent risk factor increasing the probability of graft loss by 10%. Other classical independent factors were also identified in this analysis. The difference in graft survival was mainly observed within the early posttransplant period and was thereafter stable on the long term, except for HCC and partial grafts for which the difference enlarged beyond the first and second year of follow up.

While initial studies comparing HTK and UW were unable to demonstrate any statistical difference in liver graft survival, possibly because of the limited number of patients, more recently a study made on a large sample size based on UNOS data and comparing HTK with UW, identified HTK as an independent risk factor of graft loss. This was observed especially with donation after cardiac death (DCD) allografts and those with cold ischemia time over 8 h (13). The authors found indeed a disproportionate use of HTK in DCD recoveries in recent years likely because it was thought that the lower viscosity of HTK would be particularly beneficial during a DCD procurement. Overall, these results were observed even considering that HTK livers had shorter cold ischemia time and their recipients showed better characteristics in relation to the rate of pretransplant hospitalization, life support prior to liver transplant.

The present study based on the ELTR, and powered with a significant sample size allowing reliable multivariate analysis is in agreement with these results. At the difference of the UNOS study, the proportion of DCD donors since 2008 in Europe was only 2.8% (2.4% for HTK vs. 2.9% for the other solutions [NS]), suggesting that HTK effect was independent of this factor. Noteworthy, the deleterious

Table 3: Univariate analysis of graft survival

Variable	Option	N	1 year	3 years	5 years	8 years	10 years	p
Recipient age	15–45 years	9634	83%	76%	71%	66%	63%	<0.0001
	45–60 years	22 106	82%	74%	69%	62%	55%	
	≥60 years	11 052	79%	70%	64%	56%	49%	
Recipient HBsAg	Negative	34 441	81%	73%	68%	61%	54%	<0.0001
	Positive	5099	85%	79%	75%	70%	68%	
Recipient anti-HCV	Negative	28 179	83%	76%	71%	65%	59%	<0.0001
	Positive	11 161	81%	69%	62%	54%	46%	
Recipient HIV	Negative	31 183	82%	74%	68%	61%	53%	<0.0001
	Positive	588	77%	64%	59%	47%	–	
Urgency	No	33 659	82%	74%	68%	62%	56%	<0.0001
	Yes	3238	72%	66%	62%	59%	59%	
UNOS status*	1	3307	69%	64%	61%	58%	57%	<0.0001
	2	5788	76%	69%	64%	56%	52%	
	3	18 436	83%	75%	69%	63%	55%	
	4	10 242	85%	78%	72%	65%	59%	
Preservation	Celsior	7749	82%	73%	68%	59%	54%	<0.0001
Solution	HTK	8657	77%	69%	64%	57%	56%	
	IGL	1855	82%	75%	68%	66%	–	
	UW	24 531	83%	75%	69%	63%	56%	
Ischemia time	[1–6 h]	9781	84%	76%	71%	65%	64%	<0.0001
	[6–12 h]	26 227	82%	74%	68%	61%	54%	
	[12–18 h]	4065	76%	70%	63%	56%	48%	
	[18–36 h]	104	78%	68%	66%	66%	66%	
Type of graft	Cad. Full size	37 428	82%	74%	69%	62%	55%	<0.0001
	Domino	473	81%	70%	66%	45%	42%	
	Living	2547	79%	72%	66%	61%	59%	
	Reduced	97	68%	62%	52%	52%	–	
	Split	1483	74%	68%	64%	56%	54%	
Main disease	AHF	2653	72%	67%	64%	60%	60%	<0.0001
	Cancer	8555	83%	70%	62%	54%	46%	
	Cirrhosis	25 333	82%	75%	69%	63%	57%	
	Others	5829	82%	76%	72%	65%	59%	
LT1 donor age ≥65 years	Yes	8045	80%	69%	62%	54%	47%	<0.0001
	No	30 808	82%	75%	70%	63%	57%	
ABO blood group	Compatible	2435	77%	70%	66%	62%	58%	<0.0001
	Identical	39 170	82%	74%	68%	62%	55%	
	No identical	259	69%	62%	59%	50%	–	
No LT center <500	No	34 655	82%	74%	68%	61%	56%	0.008
	Yes	8137	80%	72%	67%	62%	52%	

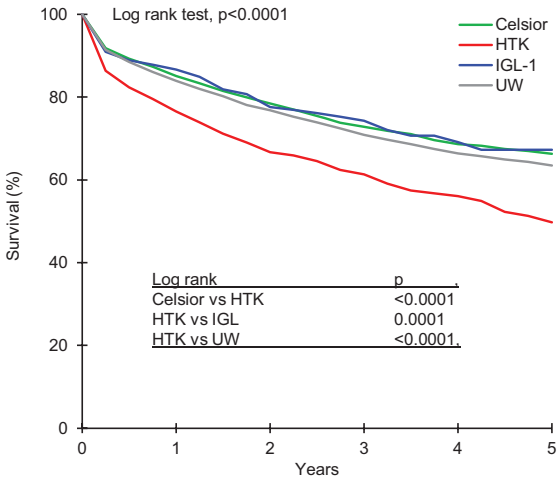
*(1) ICU-bound, (2) Continuous hospitalization, (3) Continuous medical care, (4) At home with normal function.

effect of HTK was observed independently of the duration of ischemia time: both studies evidenced that for ischemia time exceeding 6 h HTK preservation experienced the lowest graft survival when compared to the other solutions. Also, in one of the largest single-center study reported by Mangus et al (14), a trend toward reduced survival was observed for HTK preserved livers suffering from more than 12 h of cold ischemia time. In the evaluation of subgroups focused on HCC and on partial grafts, factors well known to be associated to reduced survival, the risk of using HTK preservation solution was confirmed.

These limitations of HTK were also reported by Nardo et al (15) when compared to CE, suggesting a lower survival with the use of HTK. Our study is in line with these results and lower graft survivals were achieved with HTK compared to CE in the global analysis of all transplants, in HCC recipients and also in partial grafts.

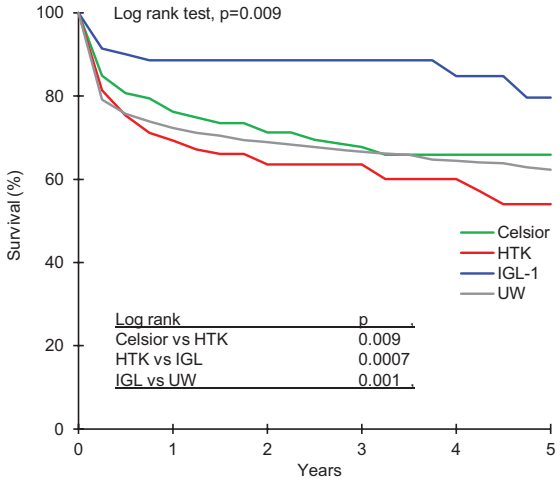
Concerning the comparison between HTK and IGL-1, to our knowledge there was no published data and our study is the first one to report significantly better results for IGL-1.

Regarding IGL-1 and UW, Dondéro et al (16) reported equivalent clinical results in a prospective randomized study including 140 patients from a single-center trial. This equivalence was confirmed in the current study with regard to graft survival since IGL-1 shows similar graft survival than UW in the global cohort as well as in the subgroup analyses. Graft survival with IGL-1 exceeds however UW in case of LT with partial grafts.



	1 yr	2 yrs	3 yrs	4 yrs	5 yrs
Celsior	85%	78%	73%	69%	66%
HTK	76%	67%	61%	56%	50%
IGL	87%	78%	74%	69%	67%
UW	84%	77%	71%	66%	63%
Exposed patients					
Celsior	1481	1051	759	530	346
HTK	777	492	341	214	126
IGL	219	116	73	43	20
UW	2964	2379	1862	1474	1100

Figure 4: Graft survival after liver transplantation according to preservation solution. Sub-group of HCC patients.



	1 yr	2 yrs	3 yrs	4 yrs	5 yrs
Celsior	76%	71%	68%	66%	66%
HTK	69%	64%	64%	60%	54%
IGL	89%	89%	89%	85%	80%
UW	72%	69%	67%	64%	62%
Exposed patients					
Celsior	114	92	74	55	33
HTK	70	48	37	21	17
IGL	55	36	26	22	14
UW	581	497	431	364	294

Figure 5: Graft survival after liver transplantation according to preservation solution. Sub-group of patients receiving grafts (split or reduced).

Table 4: Multivariate analysis of risk factors for graft loss in the global cohort. Center stratified Cox regression analysis

Risk factors	p	RR	CI 95%
1. Recipient HIV (+)	<0.0001	1.50	[1.29; 1.75]
2. Donor age ≥65 years	<0.0001	1.41	[1.32; 1.51]
3. Recipient anti HCV (+)	<0.0001	1.40	[1.34; 1.47]
4. Main disease: ACHF	<0.0001	1.34	[1.22; 1.47]
5. Partial liver graft	<0.0001	1.30	[1.16; 1.44]
6. Recipient age ≥60 years	<0.0001	1.29	[1.23; 1.37]
7. Non-ABO isogroup	<0.0001	1.24	[1.14; 1.34]
8. Recipient HBsAg (−)	<0.0001	1.24	[1.15; 1.33]
9. Ischemia time ≥12 h	<0.0001	1.19	[1.11; 1.27]
10. Recipient male	<0.0001	1.10	[1.05; 1.15]
11. HTK	0.02	1.10	[1.01; 1.20]
12. Main disease: not cirrhosis	0.01	1.09	[1.04; 1.15]

RR, risk ratio; CI, confidence interval; ACHF, acute hepatic failure. Cox model with 34 520 observations.

The mechanisms by which HTK leads to a worst overall preservation of the liver are not fully understood. We could hypothesize that it could be related to viscosity, to the oncotic value of the solution or to the presence of antioxidant agents (3). In principle lower viscosity favors an efficient organ washout and the diffusion of preservation solution to the distal organ tissue, limiting biliary complications well described to occur after transplantation (17). The benefit of a lower viscosity has been documented in an experimental model of LT in rats in which an initial flush with HTK before UW preservation (being UW more viscous than HTK) improved enzyme release and bile production after transplantation (18). However, the efficiency of the solution is also strongly dependent on the presence of antioxidants and protective agents. HTK composition is poor in these agents when compared to the other solutions. Another factor such as the oncotic agent plays a key role in preventing tissue edema, and HTK does not contain it when compared to IGL-1 polyethylene glycol 35 kDa [PEG-35] and UW hydroxyethyl starch [HES] (19). Limitation of interstitial edema becomes crucial in the cold preservation of organs, especially liver and pancreas where this edema is determinant for limiting organ function after reperfusion (20). Furthermore, in steatotic livers the microcirculation through the sinusoids is reduced as a consequence of the fat accumulated in hepatocytes that increase their volume collapsing the sinusoidal vascular space. In this situation an important edema would even worsen and reduce the vascular space impeding the perfusion of the organ. Thus, reduction of edema in steatotic livers with HES or PEG-35 should improve the microcirculation and the preservation of the organ through the efficient flushing with the preservation solution (21). These benefits of the oncotic agent have been extensively described on fatty livers experimentally (2) and should be balanced by the compromise of providing an acceptable low viscosity to the solution. In our study, since the steatosis item was not available in the ELTR questionnaire before 2008, this variable could not be evaluated.

The superior benefits of CE versus HTK could be related to the different composition and presence of protective agents, in favor of CE, provided that neither contain oncotic agents. This is supported by *in vitro* studies demonstrating the superior protection conferred by CE as previously reported by several authors. In this sense, CE induced a superior protection against preservation injury of human liver endothelial cells in culture in terms of LDH and maintenance of energy metabolism after reperfusion (22). This is also consistent with the report by Straatsburg et al (23) showing in an isolated rat liver model that rat livers

Table 5: Cause of death or graft loss

Preservation solution N	CE (%) 7756	HTK (%) 8696	IGL (%) 1855	UW (%) 24 562	p
Infection	12.9	19.8	16.9	13.3	<0.0001
Vascular	8.7	5.9	4.9	9.1	<0.0001
Nontumoral reccurence	13.8	4.6	9.8	11.8	<0.0001
Intaoperative death	2.1	1.7	0.6	1.8	NS
Tumoral reccurence	8.1	10.5	3.7	9.6	0.0004
Others	6.8	6.5	8.3	7.2	NS
PNF or dysfunction	8.3	13.7	7.7	7.1	<0.0001
Cardiovascular	5.6	7.1	7.9	5.8	NS
Tumor *de novo*	4.5	3.0	5.5	7.6	<0.0001
Rejection	4.0	2.3	3.4	4.7	0.0001
Biliary	2.8	6.0	3.1	4.2	<0.0001
Pulmonary	4.9	3.5	4.0	3.9	NS
Other hepatic	3.4	5.3	3.7	3.5	0.005
Cerebrovascular	4.2	2.8	3.7	2.8	0.04
GI	2.7	2.8	1.5	2.2	NS
Renal	0.7	1.2	1.0	1.0	NS
Hemorrhage	0.8	0.9	3.1	0.5	<0.0001
Hepatic infarction	1.3	0.0	0.6	0.2	<0.0001

were better preserved in CE than HTK with increased bile secretion and reduced cell death. Also in a model of isolated porcine hepatocyte preservation, CE induced less necrosis and DNA fragmentation than HTK (24).

Interestingly, in partial grafts, IGL-1 provided better survival than the other solutions. This could be explained by recent experimental studies showing the relation between this solution and the increase of mediators promoting liver regeneration such as AMP-activated protein kinase, mechanisms not induced by UW (25). This fact should be considered given the potential benefits of activation of regeneration mechanisms in partial graft LT.

This study has some limitations. It is retrospective and nonexhaustive of all the data concerning particularly the early posttransplant course. The different groups presented some differences concerning donors, recipients, indications or details of the operative procedure. However, the large multicentric cohort of patients prospectively collected through the ELTR, allowed a multivariate as well as different subgroup analyses, converging in a robust evaluation of the influence of preservation solutions on graft outcome. Also, owing to the 10-year period of the study, a ''time effect'' could be suspected to impact the graft survival, with a wider use of new solutions in the recent period. This was not the case since a very small difference in graft survival was observed between the period 2003–2007 and that of 2008–2012 (1-year graft survival 82% vs. 81%, respectively) paradoxically in favor of the oldest period. Therefore the time period has not affected the independent effect of HTK. Finally any center effect on graft outcome was excluded by the stratification of the Cox regression model.

In conclusion, the experience here reported from the ELTR clearly shows that preservation with HTK is an independent factor of graft loss after transplantation. Detailed analysis of the data suggests that UW and IGL-1 provide the best graft survival with CE results being close. The differential effects of the solutions are attributable to their different compositions raising the importance of each component on the preservation capacity of the solutions available. These results may serve as a basis to design future prospective randomized clinical trials.

Acknowledgments

The authors thank Marc Net for his help with the preparation of this manuscript.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

References

1. Casillas-Ramírez A, Mosbah IB, Ramalho F, Roselló-Catafau J, Peralta C. Past and future approaches to ischemia–reperfusion lesion associated with liver transplantation. Life Sci 2006; 79: 1881–1894.
2. Zaouali MA, Ben Abdennebi H, Padrissa-Altés S, Mahfoudh-Boussaid A, Roselló-Catafau J. Pharmacological strategies against total ischemia reperfusion injury. Expert Opin Pharmacother 2010; 11: 537–555.
3. Lee CY, Mangino MJ. Preservations methods for kidney and liver. Organogenesis 2009; 5: 105–112.
4. O'Callagan JM, Morgan RD, Night SR, Morris PJ. Systematic review and meta-analysis of hypothermic machine perfusion versus static cold storage of kidney allografts on transplantation outcomes. Br J Surg 2013; 100: 991–1001.
5. Guarrera JV, Henry SD, Samstein B, et al. Hypothermic machine preservation in human liver transplantation: The first clinical series. Am J Transplant 2010; 10: 372–381.
6. Ferrigno A, Rizzo V, Boncompagni E, et al. Machine perfusion at 20°C reduces preservation damage to livers from non-heart beating donors. Cryobiology 2011; 62: 152–158.
7. Schlegel A, Graf R, Clavien PA, Dutkowski P. Hypothermic oxygenated perfusion (HOPE) protects from biliary injury in a rodent model of DCD liver transplantation. J Hepatol 2013; 59: 984–991.
8. Jamieson RW, Zilvetti M, Roy D, et al. Hepatic steatosis and normothermic perfusion-preliminary experiments in a porcine model. Transplantation 2011; 92: 289–295.
9. Fondevila C, Hessheimer AJ, Maathuis MH, et al. Superior preservation of DCD livers with continuous normothermic perfusion. Ann Surg 2011; 254: 1000–1007.
10. O'Callaghan JM, Morgan RD, Knight SR, Morris PJ. The effect of preservation solutions for storage of liver allografts on transplant outcomes: A sytematic review and meta-analysis. Ann Surg 2014; 260: 46–55.
11. Karam V, Gunson B, Roggen F, et al. Quality control of the European Liver Transplant Registry: Results of audit visits to the contributing centers. Transplantation 2003; 75: 2167–2173.
12. Adam R, McMaster P, O'Grady JG, et al. Evolution of liver transplantation in Europe: Report of the European Liver Transplant Registry. Liver Transpl 2003; 9: 1231–1243.
13. Stewart ZA, Cameron AM, Singer AL, Montgomery RA, Segev DL. Histidine-tryptophan-ketoglutarate (HTK) is associated with reduced graft survival in deceased donor livers, especially those donated after cardiac death. Am J Transplant 2009; 9: 286–293.
14. Mangus RS, Fridell JA, Vianna RM, et al. Comparison of histidinetryptophan-ketoglutarate solution and University of Wisconsin solution in extended criteria liver donors. Liver Transpl 2008; 14: 365–373.
15. Nardo B, Bertelli R, Montalti R, et al. Preliminary results of a clinical randomized study comparing Celsior and HTK solutions in liver preservation for transplantation. Transplant Proc 2005; 37: 320–322.
16. Dondéro F, Paugam-Burtz C, Danjou F, Stocco J, Durand F, Belghiti J. A randomized study comparing IGL-1 to the University of Wisconsin preservation solution in liver transplantation. Ann Transplant 2010; 15: 7–14.
17. Feng XN, Xu X, Zheng SS. Current status and erspective of liver preservation solutions. Hepatobiliary Pancreat Dis Int 2006; 5: 490–494.
18. Puhl G, Olschewski P, Schoning W, et al. Low viscosity histidine-tryptophan-ketoglutarate graft flush improves subsequent extended cold storage in University of Wisconsin solution in an

extracorporeal rat liver perfusion and rat liver transplantation model. Liver Transpl 2006; 12: 1841–1849.
19. Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. Transplantation 1988; 45: 673–676.
20. 't Hart NA, Leuvenink HG, Ploeg RJ. New solutions in organ preservation. Transplant Rev 2002; 16: 131–141.
21. Ramalho FS, Fernandez-Monteiro I, Rosello-Catafau J, Peralta C. Hepatic microcirculatory failure. Acta Cir Bras 2006; 1: 48–53.
22. Janssen H, Janssen PH, Broelsch CE. UW is superior to Celsior and HTK in the protection of human liver endothelial cells against preservation injury. Liver Transpl 2004; 10: 1514–1523.
23. Straatsburg IH, Abrahamse SL, Song SW, Hartman RJ, Van Gulik TM. Evaluation of rat liver apoptotic and necrotic cell death after cold storage using UW, HTK, and Celsior. Transplantation 2002; 74: 458–464.
24. Abrahamse SL, van Runnard Heimel P, Hartman RJ, Chamuleau RA, van Gulik TM. Induction of necrosis and DNA fragmentation during hypothermic preservation of hepatocytes in UW, HTK, and Celsior solutions. Cell Transplant 2003; 12: 59–68.
25. Padrissa-Altés S, Zaoualí MA, Roselló-Catafau J. AMP-activated protein kinase as a target for preconditioning in transplantation medicine. Transplantation 2010; 90: 1241.